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Long-term observation of neuronal degeneration and microgliosis in the gerbil dentate gyrus after transient cerebral ischemia

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ABSTRACT

Ischemic insults in the central nervous system evoke activation of microglia. In this study, we investigated long-term changes of neuronal damage and microglial activation in the gerbil dentate gyrus for 60 days after transient cerebral ischemia using immunohistochemistry and western blot. Neuronal damage or death was hardly found in the dentate gyrus after transient ischemia using cresyl violet staining and NeuN immunohistochemistry; however, neuronal degeneration was detected in the polymorphic layer of the dentate gyrus using Fluoro-Jade (F-J) B staining. F-J B-positive cells were significantly increased after ischemia-reperfusion (I-R) and peaked at 3 days post-ischemia, thereafter, F-J B-positive cells were decreased in a time-dependent manner and shown until 30 days post-ischemia; no F-J B-positive cells were observed 60 days after I-R. On the other hand, Iba-1-immunoreactive microglia were hypertrophied after I-R, and numbers of Iba-1-immunoreactive microglia were significantly increased along with the neuronal degeneration and highest 7 days after I-R, thereafter, numbers of Iba-1-immunoreactive microglia were decreased with time, although microglia activation lasted up to 60 days after I-R. In addition, Iba-1 protein level in the dentate gyrus after I-R was changed like immunohistochemical change. Our results, in brief, indicate that transient ischemia-induced neuronal degeneration in the dentate gyrus is maintained for about 30 days after I-R and that microglial activation lasts up to, at least, 60 days after I-R in the gerbil dentate gyrus after transient cerebral ischemia.

1. Introduction

Transient cerebral ischemia causes neuronal damage of vulnerable neurons in some selective brain regions such as the hippocampus [1, 2]. The hippocampal subregions have different vulnerability to transient cerebral ischemia; pyramidal cells in the hippocampal CA1 region and polymorphic cells in the hippocampal dentate gyrus are most vulnerable to transient ischemia [1, 3], although pyramidal cells in the CA3 region and granule cells in the dentate gyrus are comparatively resistant to transient ischemia [4, 5]. In addition, polymorphic cells in the dentate gyrus are earlier degenerated than CA1 pyramidal cells, which are dead 4–5 days after transient cerebral ischemia [6].

Neuroinflammation may be initiated in response to infection, injuries, toxic metabolites, or autoimmunity [7–9]. In the central nervous system, microglia are the resident inflammatory cells that are activated in response to these cues [7]. It has been reported that microglia respond rapidly to ischemic stroke and activated microglia contribute to neuronal vulnerability because they are able to synthesize and release immunomodulators such as cytokines, chemokines [10, 11]; in contrary, activated microglia are involved in the clearance of damaged cells and the secretion of neurotrophins, which is a sign of disease-induced adaptation [12]. Furthermore, inflammatory process shows very different cellular events with time after ischemic stroke [13].

As described above, transient ischemia-induced neuronal damage/death and microgliosis in the hippocampal CA1 region has been demonstrated; however, long-term changes of neuronal degeneration and microglial activation in the dentate gyrus of ischemic brain have not

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yet defined. Therefore, the purpose of the present study was to investigate neuronal degeneration as well as microglial responses in the dentate gyrus over a 60-day period after transient cerebral ischemia in the gerbil, which is a useful animal model for transient cerebral ischemia [14].

2. Materials and methods

2.1. Experimental animals

Male Mongolian gerbils (*Meriones unguiculatus*) were obtained from the Experimental Animal Center, Kangwon University, Chuncheon, South Korea. Gerbils were used at 6 months (B.W., 66–75 g) of age. The animals were housed in a conventional state under adequate temperature (23 °C) and humidity (60%) control with a 12-h light/12-h dark cycle, and provided with free access to water and food. The procedures for animal handling and care adhered to guidelines that are in compliance with the current international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, The National Academies Press, 8th ed., 2011), and they were approved by the Institutional Animal Care and Use Committee (IACUC) at Kangwon National University (approval no. KW-130424-1). All experiments were conducted to minimize the number of animals used and avoid animal suffering.

2.2. Induction of transient cerebral ischemia

In brief, according to our published procedure [15], the animals were anesthetized with a mixture of 2.5% isoflurane (Baxtor, Deerfield, IL) in 33% oxygen and 67% nitrous oxide. Bilateral common carotid arteries were occluded for 5 min using non-traumatic aneurysm clips. The body (rectal) temperature under free-regulating or normothermic (37 ± 0.5 °C) conditions was monitored with a rectal temperature probe (TR-100; Fine Science Tools, Foster City, CA) and maintained using a thermometric blanket before, during and after the surgery until the animals completely recovered from anesthesia. Sham-operated animals were subjected to the same surgical procedures except that the common carotid arteries were not occluded.

2.3. Tissue processing for histology

To investigate neuronal degeneration and microglial activation after transient cerebral ischemia, hippocampal tissue sections from the sham- and ischemia-operated animals ($n = 7$ in each group) were used at designated times (1, 3, 5, 7, 10, 15, 30, and 60 days) after the ischemic surgery. In brief, according to the method of our previous study [15], the animals were anesthetized with sodium pentobarbital and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate-buffer (PB, pH 7.4). The brain tissues were cryoprotected and serially sectioned on a cryostat (Leica, Wetzlar, Germany) into 30-μm coronal sections, and they were then collected into six-well plates containing PBS.

2.4. Cresyl violet (CV) staining

To investigate cellular changes after ischemia, CV staining was performed. In brief, according to the method of our previous study [15], the sections were mounted on gelatin-coated microscopy slides. Cresyl violet acetate (Sigma, MO) was dissolved at 1.0% (w/v) in distilled water, and glacial acetic acid was added to this solution. The sections were stained and dehydrated by immersing in serial ethanol baths, and they were then mounted with Canada balsam (Kanto, Tokyo, Japan).

2.5. Immunohistochemistry

According to the method of our previous study [15], in brief, immunohistochemical staining was performed using mouse anti- neuronal nuclei (NeuN, 1:1000, Chemicon International, Temecula, CA) for neurons and rabbit anti-Iba-1 (1:800, Wako, Osaka, Japan) for microglia as the primary antibodies, and biotinylated horse anti-mouse IgG or goat anti-rabbit IgG (1:200, Vector, Burlingame, CA) and streptavidin peroxidase complex (1:200, Vector) as the secondary antibodies. A negative control test was carried out using pre-immune serum instead of primary antibody in order to establish the specificity of the immunostaining, and the negative control resulted in the absence of immunoreactivity in all structures.

2.6. Fluoro-Jade (F-J) B histofluorescence staining

To investigate neuronal death after transient cerebral ischemia, F-J B (a high affinity fluorescent marker for the localization of neuronal degeneration) histofluorescence staining was conducted according to the method of our previous study [15]. In brief, the sections were first immersed in a solution containing 1% sodium hydroxide in 80% alcohol, and followed in 70% alcohol. They were then transferred to a solution of 0.06% potassium permanganate, and transferred to a 0.0004% F-J B

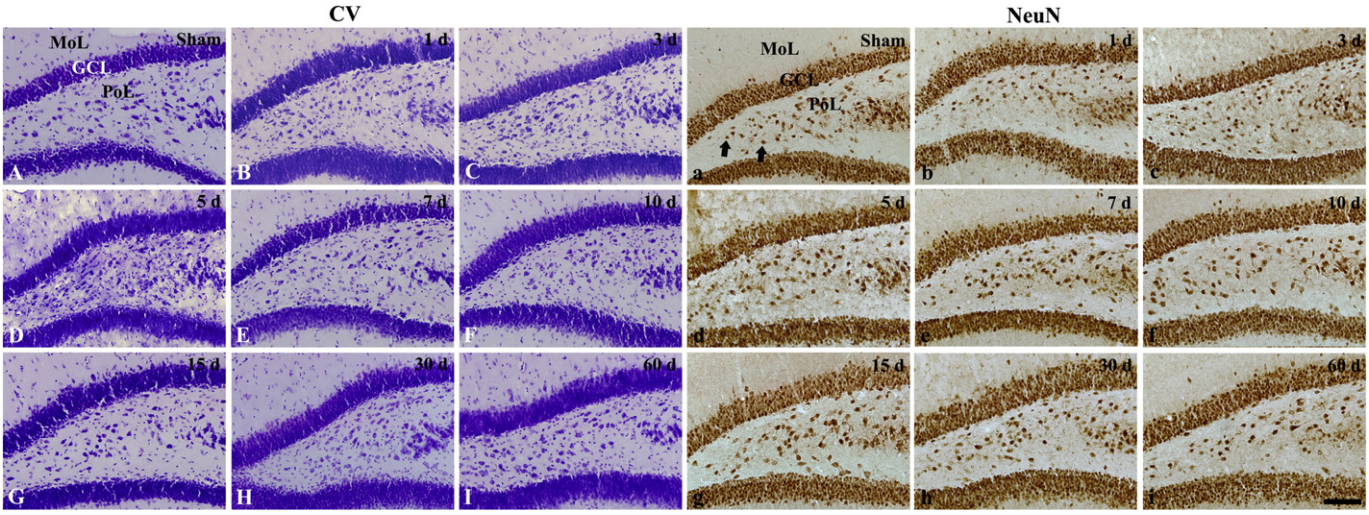

Fig. 1. CV staining (A–I) and NeuN immunohistochemistry (a–i) in the dentate gyrus of the sham-operated- (A and a) and ischemia- (B–I and b–i) groups. The distribution pattern of CV-positive cells are not changed after ischemia; however, the number of NeuN-immunoreactive neurons (arrows) in the polymorphic layer (PoL) shows a tendency to decrease with time after ischemia. GCL, granule cell layer; MoL, molecular layer. Scale bar = 100 μm.